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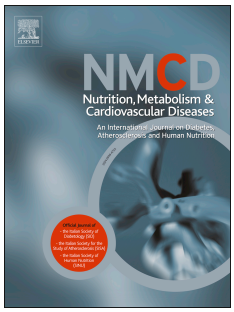

Association between lipids and apolipoproteins on type 2 diabetes risk; moderating effects of gender and polymorphisms; the ATTICA study

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PII: S0939-4753(20)30024-7

DOI: https://doi.org/10.1016/j.numecd.2020.01.008

Reference: NUMECD 2213

To appear in: *Nutrition, Metabolism and Cardiovascular Diseases*

Received Date: 20 September 2019

Revised Date: 14 November 2019

Accepted Date: 7 January 2020

Please cite this article as: Mellor DD, Georgousopoulou EN, D'Cunha NM, Naumovski N, Chrysohoou C, Tousoulis D, Pitsavos C, Panagiotakos DB, the ATTICA Study Group, Association between lipids and apolipoproteins on type 2 diabetes risk; moderating effects of gender and polymorphisms; the ATTICA study, *Nutrition, Metabolism and Cardiovascular Diseases*, https://doi.org/10.1016/j.numecd.2020.01.008.

Association between lipids and apolipoproteins on type 2 diabetes risk; moderating effects of gender and polymorphisms; the ATTICA study.

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Declarations of interest: None.

Abstract word count: 250

Word count: 3827

Tables: 3

Figures: 0

Highlights

- ApoA1 levels, ApoB:LDL and TG:apoA1 ratios are associated with 10-year risk of T2DM in males only
- In males only a unit change in apoB: LDL cholesterol increased risk of type 2 diabetes by 303%
- In males only a unit change in triglycerides: apoA1 increased risk of type 2 diabetes by 85%
- HOMA-IR predicted the 10-year incidence of T2DM only in apoA1 75 GG carriers
- Physical activity may moderate the influence of HOMA-IR on T2DM incidence only in carriers of apoA1 75 GG.

Abstract

Background and Aims: Type 2 diabetes mellitus (T2DM) is a condition defined by hyperglycaemia, but also often presents with dyslipidaemia and suppressed HDL cholesterol. Mendelian randomization studies have suggested a causal link between low HDL cholesterol and T2DM. However, influences of gender, polymorphisms and lifestyle, all known to influence HDL cholesterol, have not been fully explored in a prospective cohort.

Methods and Results: In 2001-2002, a random sample of 1514 males (18-87 years old) and 1528 females (18-89 years old) were recruited in the ATTICA study. The 10-year follow-up (2011-2012) included 1485 participants. Lipids and lipoproteins levels, glucose and insulin levels were measured together with apolipoprotein A1 (apoA1) 75 G/A genotype, which is known to influence HDL-cholesterol. In total, 12.9% of the study sample developed T2DM within the 10-year follow-up period. In multivariable models, for each mg/dL increase in apoA1 levels in males, 10-year T2DM risk decreased 1.02%; while every unit increase in apoB/LDL-cholesterol ratio increased risk 4-fold. Finally, for every unit increase in triglycerides/apoA1 ratio, the risk increased 85%. HOMA-IR independently predicted T2DM 10-year incidence only for carriers of GG polymorphism (all, $p<0.05$), but not in carriers of the GA polymorphism (all, $p>0.05$).

Conclusion: ApoA1 was associated with decreased T2DM risk and TG/ApoA1 and apoB/LDL were associated with increased risk of T2DM, only in males. ApoA1 polymorphism, which is associated with lower HDL cholesterol, influenced the predictive effects of HOMA-IR on T2DM incidence, which appeared to be moderated by physical activity, suggesting potential scope for more targeted preventative strategies.

Keywords: Lipids; HDL cholesterol; Apolipoprotein A-1; Type 2 Diabetes Risk; Prospective cohort.

Abbreviations:

apoA1: Apolipoprotein A-1

apoB: Apolipoprotein B

BMI: Body mass index

CVD: cardiovascular disease

HDLc: High-Density Lipoprotein cholesterol

IPAQ: International Physical Activity Questionnaire

LDLc: Low-Density Lipoprotein cholesterol

TG: Triglycerides

T2DM: Type 2 Diabetes Mellitus

Introduction

Type 2 diabetes mellitus (T2DM) is a rapidly growing global health challenge [1-3]. Traditionally, management and prevention of T2DM have focused mainly on glycaemia [4, 5], despite it often presenting with dyslipidaemia. It is plausible that after hyperinsulinaemia, dyslipidaemia typified by suppressed HDL cholesterol is the second most dominant feature of Metabolic Syndrome in T2DM [6]. Recently, a Mendelian randomisation study suggested a potential causal link between suppressed HDLc and T2DM risk [7]. Despite this emerging evidence, the use of lipid and lipoprotein biomarkers still mainly focuses upon predicting cardiovascular disease (CVD) risk [8, 9] including in people with T2DM [10], with little consideration of these biomarkers when assessing T2DM risk [11].

The mechanisms of how apolipoproteins and HDLc influence insulin action, a key aspect of T2DM pathogenesis has recently been explored [12]. The relationship between low HDLc and development of T2DM has been previously described [13, 14], with causality partially assessed [15] as plasma insulin increased and plasma glucose decreased following an infusion of HDLc (including apoA1) in individuals with T2DM. The role of HDLc at physiological levels is less clear and dependent on experimental conditions. However, it appears that HDLc is potentially protective of β-cells against stressors, including glucose and oxidised LDLc [16, 17], whereas triglyceride-rich particles may be detrimental [18]. The protective effects of HDLc may be attributable to apoA1 [17]; whereas low levels of HDLc are a known risk factor [12].

There are several known modifiers of cardiovascular risk including gender, genetic factors and lifestyle which appear to act via altering an individual's lipid profile [19], which may also influence the risk of developing T2DM. Males typically have lower HDLc compared to pre-menopausal females, leading to suggestions that CVD risk reduction strategies should be tailored accordingly [20]. Beyond gender, a number of polymorphisms

have been identified in apolipoprotein genes. A key single nucleotide polymorphism in the apoA1 gene is apoA1-75 G/A, which is associated with a lower apoA1 and HDLc concentration for the GG genotype compared to the AA and a lesser extent GA [21]. The G allele has also been associated with increased myocardial infarction risk [22]. It is, therefore, logical to explore the potential moderating effects of this polymorphism in cohorts at risk of developing T2DM.

Although HDLc has been proposed to be linked causally with T2DM; the link with and apoA1 and T2DM is less well defined, especially with respect to modifying effects of gender, polymorphisms or lifestyle. Therefore, this study aimed to explore potential associations of lipids, apolipoproteins, gender and apoA1 polymorphisms, and risk of developing T2DM in a cohort of Greek healthy adults.

Materials and methods

Baseline sampling procedure (2001-2002)

The ATTICA study is a large-scale, health and nutrition, prospective survey, which was carried out during 2001-2002, in the province of Attica, where Athens is a major metropolis. People with a history of CVD or other atherosclerotic diseases or having chronic viral infections or living in institutions were excluded from participation. Of the initially invited 4056 individuals and after excluding those with CVD (i.e., n=117) or those having chronic viral infections (n=107), 3042 finally agreed to participate (75% participation rate); 1514 of the participants were male (aged 46±13 y; range 18-87 y), and 1528 were female (aged 45±13 y; range: 18–89y). Trained personnel (i.e., cardiologists, general practitioners, dietitians and nurses) interviewed the participants, using a standard questionnaire.

More details about the aims, design and methods used in the ATTICA Study may be found elsewhere in the literature [23].

Baseline measurements

Baseline assessment included information about socio-demographic characteristics (age and gender), history of diabetes, family history of diabetes, smoking status and physical activity. Smokers were defined as those who smoked at least one cigarette per day or had quitted within the previous year; the rest were defined as non-smokers. The International Physical Activity Questionnaire (IPAQ) was used to evaluate the level of physical activity [24]. Participants were classified into two groups; either sedentary lifestyle or at least moderately active during a substantial part of the day. Weight (kg), height (m), as well as clinical characteristics, were measured using standardized procedures. Body mass index (BMI) was calculated as weight (kg) divided by standing height (in square meters).

Biochemical measurements were carried out in the same laboratory that followed the criteria of the World Health Organization Lipid Reference Laboratories. Blood samples were collected from the antecubital vein between 8, and 10 am, in a sitting position after 12 hours of fasting and alcohol abstinence. Blood glucose levels (mg/dl) were measured with a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA, USA). Serum insulin concentrations were assayed by radioimmunoassay (RIA100, Pharmacia Co., Erlangen, Germany). Insulin resistance was assessed by the calculation of the homeostasis model assessment (HOMA-R) approach (glucose in mg/dl x insulin in μU/ml / 22.5) [25]. Diagnosis of T2DM was based on the criteria of the American Diabetes Association (ADA) [26], i.e., participants who had fasting blood glucose >125 mg/dl during the examination or who reported the use of antidiabetic medication were defined as having diabetes. Serum total

cholesterol, HDL-cholesterol and triglycerides were measured using chromatographic enzymic method in a Technicon automatic analyser RA-1000 (Dade Behring, Marburg, Germany). HDL-cholesterol was determined after precipitation of the Apolipoprotein B (apoB) containing lipoproteins with dextran-magnesium-chloride. Non-HDL cholesterol was calculated by the formula: total cholesterol minus HDL cholesterol. Lipoprotein (a) was measured by a latex-enhanced turbidimetric immunoassay. LDL cholesterol calculated using the Friedewald formulae: (8) – [13] – 1/5 (triglycerides) (only for participants with triglycerides < 400 mg/dL). apoB and apoAI were measured by rate immunonephelometry. Internal quality control was in place for assessing the validity of cholesterol, triglyceride and HDL methods. The intra and inter-assay coefficients of variation of cholesterol levels did not exceed 9 %, triglycerides 4 % and HDL 4 %. Serum for the measurement of blood lipids was harvested immediately after admission.

The combination of ratios used in the analysis was based on that published previously in association with cardiovascular risk [27, 28]. Comparing the cholesterol marker with its corresponding apolipoprotein was selected as it has been shown to be a surrogate of function of the apolipoprotein, which would not be the case for a triglyceride to apolipoprotein ratio.

DNA extraction and genotyping

Genomic DNA was extracted from 2-5 mL of fresh or frozen whole blood using standard methods (Qiamp-DNA extraction kit, QIAGEN, Hilden, Germany), as described previously (29). The coding sequence variant was a G to T substitution in exon 7 in codon 298, which alters the amino acid at this residue from Glu to Asp. Genotyping of apoA1 gene polymorphisms were performed by polymerase chain reaction (PCR) restriction length polymorphism assay previously described [29]. The location of the MspI restriction site was used to identify polymorphisms, with presence of the restriction site at -75 bp (G allele) and

at +83 bp (C allele) in the 433 bp product resulted in four fragments of 45, 66, 113 and 209 bp. The absence of the restriction site at -75 bp (A allele) resulted in three fragments of 45, 179 and 209 bp. The absence of the restriction site at +83 bp (T allele) created a larger fragment of 254 bp instead of two fragments of 45 and 209 bp [21].

10-year follow-up evaluation (2011-2012)

During 2011-2012, the 10-year follow-up was performed. Of the n=3042 participants, *n*=2583 were allocated during the follow-up (85% participation rate). A detailed evaluation of the participants' medical status was performed. Among various endpoints, development of T2DM was recorded based on diagnosis by a physician; *n*=210 patients diagnosed with diabetes at baseline and *n*=1347 participants with no data regarding diabetes status at the 10-year follow up were not included in the present analyses, yielding a working sample of *n*=1485 participants without diabetes at baseline, as presented elsewhere [30]. Further details about the baseline procedures and the 10-year follow up of the study have been presented elsewhere [31].

Statistical analysis

Incidence of diabetes was calculated as the ratio of new cases (n=191) to the total number (*n*=1485) of participants in the follow-up. Normality for continuous variables was tested through histograms and P-P plots. Normally distributed continuous variables are presented as mean values±standard deviation, not normally distributed variables are presented as median (1^{st}, 3^{rd} quartile) and categorical variables as frequencies (relative frequencies). Associations between categorical variables were tested using chi-squared test. Comparisons of mean values of normally distributed variables between those groups were performed using Student's t-test, after ensuring equality of variances using Levene's test. For non-normally

distributed variables, the Kruskal-Wallis test was applied, and next the Mann-Whitney *test* was performed between groups. The relative risk of developing T2DM during the 10-year period according to the participants' baseline characteristics was estimated through the odds ratio (OR) and the 95% corresponding confidence interval, as derived from logistic regression models. This type of analysis was preferred since there were no accurate data about diabetes onset, but only diagnosis. Univariable logistic regression models were used to identify the variables that were possible independent predictors of T2DM onset but also known confounders (i.e., gender, age) were forced in the multivariable models and all of independent variables were tested for collinearity. Level of statistical significance was defined at α=0.05 and Bonferroni corrections were applied to all predictive models to counteract for multiple comparisons (in this case the eight models). An exploratory analysis of the influence of apoA1 polymorphism on relative risk of developing T2DM during the follow-up period was also undertaken; this included an analysis of interactions with both modifiable and non-modifiable risk factors. The SPSS version 23 (Statistical Package for Social Sciences, IBM Hellas SA, Greece) software was used for all statistical calculations.

Results

The study sample consisted of 1485 individuals (51% females) with a mean age of 45±13 years (p for gender difference>0.05). Of these, 12.9% (191) developed T2DM within the 10-year follow-up period, but no difference was detected between genders (p=0.574). Mean BMI at baseline of the total sample was 26.3±4.28kg/m^2, with males having significantly higher mean BMI than females (27.2±3.6 vs 25.3±4.7 respectively, p<0.001). Details of participant characteristics are in ***Table 1***.

T2DM incidence was not associated with gender (p=0.574), family history of T2DM (p=0.416), sedentary lifestyle (p=0.191) and age (p=0.458). Smoking was more prevalent in males than females (p<0.001). Concerning the biomarkers relating to glucose metabolism, fasting glucose, insulin and HOMA-IR were significantly lower in females than males (all p<0.001). Baseline lipoprotein and lipid biomarkers indicated that males had significantly higher total and LDLc, Triglycerides (TG) and apoB levels, but lower HDLc and apoA1 compared to females (all p<0.001), suggesting a profile highly related to gender.

Several logistic regression models were applied to investigate the net effects of different biomarkers on T2DM 10-year incidence (***Table 2***). All models were adjusted for the same set of potential confounders and stratified by gender due to significant biomarker profile differences reported at baseline (*p* for interaction <0.001) *(Table 1).* No biomarkers were associated with T2DM 10-year risk in females, but there were significant associations for males. Specifically, for each mg/dL increase in apoA1 levels in males the 10-year T2DM risk decreased per 1.1%, independently of age, smoking, physical activity status, HOMA-IR, family history T2DM and BMI. Moreover, in males for every unit increase in apoB/LDL-cholesterol ratio, the 10-year T2DM risk was 4-fold increased independent confounding risk factors used in previous models. Finally, for males, every unit increase in triglycerides/apoA1 ratio, the 10-year T2DM risk increased per 85%, independent of confounding risk factors. No other biomarker or ratios were associated with T2DM 10-year risk in males. Additionally, in this cohort, it was found that HOMA-IR was the most specific independent predictor of T2DM incidence in females, with no effect of lipids or lipoprotein as observed in males.

ApoA1-75G/A polymorphism data were available for 313 participants (GG: 215(68.7%) GA: 89(28.4%) and AA: 9(2.9%)). The AA group was therefore excluded due to its small number,

although representative of the population; this prevalence would negate any meaning being able to be derived. Polymorphism distribution was not influenced by gender (GA prevalent at 29.4% of males *(n=45)* and 29.1% of females *(n=44),* p=0.958). No association was detected between polymorphism and T2DM 10-year incidence (p=0.931). However, significant interactions were observed with apolipoprotein levels (p<0.001) that led the analysis to stratification per polymorphism group (***Table 3***).

As presented in *Table 3,* when the analysis was stratified per apoA175 G/A polymorphism status, none of the lipid or lipoprotein biomarkers were significantly related to T2DM 10-year risk (all p >0.05) after adjusting for confounding risk factors. Although influencing T2DM risk in the cohort as a whole (crude (Odds Ratio (OR) =2.44, 95% Confidence Interval (CI): 1.94-3.07), HOMA-IR was an independent predictor of 10-year incidence of T2DM, only for GG polymorphism carriers (all p<0.05) in all presented models. Contrarily, HOMA-IR was not significantly associated with T2DM 10-year risk in any of the models for carriers of the GA polymorphism (all p>0.05). Physical activity was found to be protective against T2DM only for GG carriers (Odds Ratio (OR) =0.206, 95% Confidence Interval (CI): 0.043-0.983) but not for GA carriers (OR=0.478, 95% CI: 0.033-6.84), after adjusting for confounding risk factors.

Discussion

This analysis investigated the influence of apolipoprotein and lipid biomarkers as predictive factors for developing T2DM during a 10-year follow-up, focusing on the potentially influencing effects of gender, apoA1 polymorphisms along with any interactions with insulin resistance (HOMA) and physical activity. This analysis has provided further evidence of how lipid profile and apolipoproteins influence the risk of developing T2DM in a Greek cohort

followed up for 10 years. Additionally, this is the first analysis to consider how gender and polymorphisms of apoA1-75 G/A may influence how lipoproteins and lipids influence ultimately risk of developing T2DM.

Males were found to have significantly higher total and LDLc, TG and apoB levels, but lower HDLc and apoA1 compared to females, suggesting an influence of gender upon lipid profile. However, there were no statistically significant differences in new cases of T2DM between genders. No lipid or apolipoprotein biomarkers were associated with T2DM 10-year risk in females, but associations were significant in males. Specifically, higher apoA1 levels were seen to be protective for males, while the increase in apoB/LDL-cholesterol ratio and increase in triglycerides/apoA1 ratio were aggravating factors independent of age, smoking, physical activity status, HOMA-IR, family history of diabetes and BMI.

When the analysis was stratified per apoA1-75G/A polymorphism status a known factor which influences both apoA1 and HDLc concentration, none of the biomarkers were significantly related to T2DM 10-year risk in the same multivariable models. An analysis to consider interactions between risk factors for T2DM found that HOMA-IR was an independent predictor of T2DM 10-year incidence for GG polymorphism carriers only [32]. No significant associations with T2DM 10-year risk were found in any of the models for GA polymorphism carriers.

The potential differences between genders have been largely overlooked, in a previous study which followed up a Dutch cohort for a shorter timeframe [13]. It was noticeable that the ATTICA study sample where higher risk, having a stronger family history and greater prevalence of insulin resistance than the Dutch cohort, potentially highlighting the greater

suitability of the ATTICA cohort in studying diabetes prevention [33]. A population at higher risk of developing T2DM, by virtual of increased incidence of insulin resistance as seen in this study might explain the observation in this analysis that for apoA1 to reduce risk in males, but not in females. It might also be a reflection that the Dutch cohort data was only adjusted for glucose and not insulin, so unable to adjust for insulin resistance (HOMA-IR); a known predictive of risk of developing T2DM [34, 35]. The pattern which protective effects in males of apoA1/HDLc and increasing risk from apoB/LDLc ratios were consistent with Abbasi et al. [13] and data concerning apoB/LDLc ratio additionally concurs with the male-only cohort reported by Fizelova et al. [36]. However, this is the first cohort to suggest an effect of apoA1/triglycerides as modifying risk of developing T2DM in a long-term prospective cohort. This data provides support to the logical theory; as raised triglycerides have been previously associated with increased T2DM risk [37] and insulin resistance.

The potential influencing effect of apoA1-75 G/A polymorphisms was explored in 304 participants over 10-year follow up. This is believed to be the first analysis investigating any influencing effects of this polymorphism which is known to alter lipid profiles [21], with G/G carriers expressing less apoA1 and having lower levels of HDLc [32]. Although effects of the polymorphism were not seen with respect to the risk of developing T2DM, interactions were observed with HOMA-IR only being associated with increased risk of developing T2DM in those with GG polymorphism. This suggests that identifying insulin resistance and then treating it in carriers of the GG polymorphism may provide a potentially more focused and effective intervention. The potential for targeted interventions for the prevention of T2DM was further highlighted by modulating effects of physical activity which reduced the risk of developing T2DM in GG carriers by 79% (OR 0.206 95% CI 0.043-0.983) but not in GA carriers (OR 0.478 95% CI 0.033-6.84) after adjusting for other confounding risk factors.

This warrants further investigation as it suggests that there is a gene-lifestyle interaction where physical activity may be more effective in reducing T2DM risk in GG carriers. The variation in response to exercise has been reported with greater response with a more favourable HDLc particle size with physical training for GG compared to GA or AA carriers [38]. However, to date, this effect has not been linked to the development of clinical conditions such as T2DM. Further research is needed to understand these mechanisms relative to risk of developing T2DM and effects of physical activity on the apolipoprotein, or the nature of HDLc particle size or its concentration.

Longitudinal effects of lipid and apolipoprotein measures were previously investigated in a Dutch mixed gender cohort, where gender did not influence the association between lipids, apolipoprotein markers and T2DM risk. This was despite reporting a 30% reduction in risk for males for each standard deviation shift in HDLc compared to 26% for females (OR 0.70, 95% CI 0.55-0.91 and OR 0.74 CI 0.57-0.96, respectively) [13]. A further Finish cohort of 3686 male participants completing a mean follow-up of 5.9 years [36] found an association between worsening glycaemia and incidence of T2DM linked to the ratio of apolipoprotein and its associated lipoprotein. This further supports the theory that inadequate or altered capacity of lipids by apolipoproteins could be implicated in an increased risk of developing T2DM. As an individual's lipid profile is influenced by insulin and glycaemia as well as potentially vice versa, therefore caution should be taken when looking to assign potential causality. *In vitro* work has linked apoB positively, and apoA1 negatively, to increased risk of developing T2DM [16, 17], supportive of a theory that apoA1 and HDLc are protective against the development of T2DM. However, according to our data, the nature of this effect and potential inter-individual variation appears to be further influenced by gender and apoA1-75 polymorphisms.

Clinically, the prevention of T2DM has focused on the use of glycaemia and insulin-based markers. This has been most recently seen in the commissioning of clinical services, including NHS England National Diabetes Prevention Program, which focuses purely on changes in glycaemia and weight as outcome measures [4]. Such programs focus on a glucose centric perspective of T2DM prevention despite evidence from a clinical perspective that the pathology should be increasingly seen as a global metabolic abnormality. The twin-cycle hypothesis highlights this, identifying lipid metabolism and ectopic lipid accumulation in the pancreas and liver as key drivers of the pathology [39]. This, together with experimental models and epidemiological data suggest lipids, especially HDLc and potentially HDLc/apoA1 ratio, are predictive of developing T2DM. Additionally, in an age of personalised medicine, our data suggests that recognising differences in risk associated with gender and polymorphisms could be useful in targeting interventions.

Limitations

Despite the importance of this study highlighting differences in gender and polymorphism for T2DM risk, there are a number of limitations. Firstly, the effect of gender and apoA1-75 polymorphism on lipid and apolipoprotein were only measured at baseline examination. The number of cases for the assessment of apoA1-75 polymorphism was relatively small within the whole cohort, which suggests potential bias cannot be ruled out and if the null findings are not certain although evident. The number of participants with an AA polymorphism was so small; thus, were excluded from the analysis. An alternative approach would be to combine this group with the GA. However, this did not affect the outcome. The change in lipid and apolipoprotein concentrations over the 10-year follow up was not measured, and variation could influence risk. However, this is the same methodology that has been used in

other prospective studies and is typical in this field, making results comparable. The decision to exclude individuals with a history of cardiovascular disease could potentially hinder external validity of this analysis, as it is plausible that the dyslipidaemia associated with cardiovascular disease might be protective role in type 2 diabetes incidence. However, as there is an association with type 2 diabetes and cardiovascular disease this is unlikely, and the impact of including individuals with pre-existing cardiovascular disease would, both due to the nature of the pathology and any therapeutic interventions might have would have only added additional confounding factors.

Conclusions

Markers of lipids and apolipoproteins were associated with risk of developing T2DM only in males in this Greek cohort. Additional apoA1 polymorphisms appear to influence the predictive effect of HOMA-IR on T2DM incidence and the potential moderating role of physical activity; suggesting the potential for more targeted and individualized approaches for diabetes prevention strategies based on taking into account the influencing effects of genetic factors, lipid and apolipoprotein levels.

Funding

This work was supported by the Hellenic Cardiology Society, the Hellenic Atherosclerosis Society, the Graduate Program in Applied Nutrition and Dietetics of Harokopio University and the Coca-Cola SA funded this study by research grants (KE252/ELKE/HUA). The ATTICA Study is funded by research grants from the Hellenic Society of Cardiology (grant – 1, 2002).

Declarations of interest: None.

Author Contributions

D.D.M., D.B.P., E.N.G., N.M.D., and N.N. conceptualised and wrote the paper. C.C., D.T., and C.P. interpreted the results and critically revised the manuscript. All authors approved the final version of the manuscript. D.B.P. is the guarantor of this work, had full access to all data in the study, and takes responsibility for the accuracy and integrity of the data and manuscript.

Acknowledgements

The authors would like to thank the ATTICA study group: Yannis Skoumas, Natasa Katinioti, Labros Papadimitriou, Constantina Masoura, Spiros Vellas, Yannis Lentzas, Manolis Kambaxis, Konstanitna Paliou, Vassiliki Metaxa, Agathi Ntzouvani, Dimitris Mpougatas, Nikolaos Skourlis, Christina Papanikolaou, Aikaterini Kalogeropoulou,

Evangelia Pitaraki, Alexandros Laskaris, Mihail Hatzigeorgiou, Adela Zana, Athanasios Grekas, and Eleni Kokkou, for either assistance in the physical examination and follow-up evaluation, as well as the laboratory team: Carmen Vassiliadou and George Dedousis (genetic analysis), Marina Toutouza-Giotsa, Constantina Tselika and Sia Poulopouloou (biochemical analysis) and Maria Toutouza for the database management.

436 **References**

437

438 1. World Health Organisation: **Global Report on Diabetes**. In. Geneva, Switzerland:
439 WHO; 2016: 88.
440 2. International Diabetes Federation: **International Diabetes Federation Diabetes**
441 **Atlas**. In., 7th edn. Brussels, Belgium; 2015: 144.
442 3. Polyzos SA, Mantzoros CS: **Obesity: seize the day, fight the fat**. *Metabolism* 2019,
443 **92**:1-5.
444 4. National Health Service Diabetes Prevention Programme: **NDPP National Service**
445 **Specification**. In. Edited by England N, August 2016 edn. London, U.K.: NHS
446 England 2016: 36.
447 5. Donnelly LA, Zhou K, Doney ASF, Jennison C, Franks PW, Pearson ER: **Rates of**
448 **glycaemic deterioration in a real-world population with type 2 diabetes**.
449 *Diabetologia* 2017.
450 6. Hanson RL, Imperatore G, Bennett PH, Knowler WC: **Components of the**
451 **"metabolic syndrome" and incidence of type 2 diabetes**. *Diabetes* 2002,
452 **51**(10):3120-3127.
453 7. Haase CL, Tybjærg-Hansen A, Nordestgaard BG, Frikke-Schmidt R: **HDL**
454 **Cholesterol and Risk of Type 2 Diabetes: A Mendelian Randomization Study**.
455 *Diabetes* 2015, **64**(9):3328.
456 8. Brahimaj A, Ligthart S, Ikram MA, Hofman A, Franco OH, Sijbrands EJG, Kavousi
457 M, Dehghan A: **Serum Levels of Apolipoproteins and Incident Type 2 Diabetes: A**
458 **Prospective Cohort Study**. *Diabetes Care* 2017, **40**(3):346.
459 9. Wierzbicki AS: **Further options for treating lipids in people with diabetes:**
460 **targeting LDL-cholesterol and beyond**. *Diabet Med* 2018, **35**(9):1173-1180.

10. Zhang HW, Zhao X, Guo YL, Gao Y, Zhu CG, Wu NQ, Li JJ: **Elevated lipoprotein (a) levels are associated with the presence and severity of coronary artery disease in patients with type 2 diabetes mellitus**. *Nutr Metab Cardiovasc Dis* 2018, **28**(10):980-986.

11. Berk KA, Yahya R, Verhoeven AJM, Touw J, Leijten FP, van Rossum EF, Wester VL, Lips MA, Pijl H, Timman R *et al*: **Effect of diet-induced weight loss on lipoprotein(a) levels in obese individuals with and without type 2 diabetes**. *Diabetologia* 2017, **60**(6):989-997.

12. Lee DH: **Lipoproteins and beta-Cell Functions: From Basic to Clinical Data**. *Diabetes Metab J* 2014, **38**(4):274-277.

13. Abbasi A, Corpeleijn E, Gansevoort RT, Gans RO, Hillege HL, Stolk RP, Navis G, Bakker SJ, Dullaart RP: **Role of HDL cholesterol and estimates of HDL particle composition in future development of type 2 diabetes in the general population: the PREVEND study**. *J Clin Endocrinol Metab* 2013, **98**(8):E1352-1359.

14. Fagot-Campagna A, Knowler WC, Narayan KM, Hanson RL, Saaddine J, Howard BV: **HDL cholesterol subfractions and risk of developing type 2 diabetes among Pima Indians**. *Diabetes Care* 1999, **22**(2):271-274.

15. Drew BG, Duffy SJ, Formosa MF, Natoli AK, Henstridge DC, Penfold SA, Thomas WG, Mukhamedova N, de Courten B, Forbes JM *et al*: **High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus**. *Circulation* 2009, **119**(15):2103-2111.

16. Roehrich ME, Mooser V, Lenain V, Herz J, Nimpf J, Azhar S, Bideau M, Capponi A, Nicod P, Haefliger JA *et al*: **Insulin-secreting beta-cell dysfunction induced by human lipoproteins**. *J Biol Chem* 2003, **278**(20):18368-18375.

17. Rutti S, Ehses JA, Sibler RA, Prazak R, Rohrer L, Georgopoulos S, Meier DT, Niclauss N, Berney T, Donath MY *et al*: **Low- and high-density lipoproteins modulate function, apoptosis, and proliferation of primary human and murine pancreatic beta-cells**. *Endocrinology* 2009, **150**(10):4521-4530.

18. Tricò D, Natali A, Mari A, Ferrannini E, Santoro N, Caprio S: **Triglyceride-rich very low-density lipoproteins (VLDL) are independently associated with insulin secretion in a multiethnic cohort of adolescents**. *Diabets Obes Metab* 2018, **20**(12):2905-2910.

19. Miltiadous G, Hatzivassiliou M, Liberopoulos E, Bairaktari E, Tselepis A, Cariolou M, Elisaf M: **Gene polymorphisms affecting HDL-cholesterol levels in the normolipidemic population**. *Nutr Metab Cardiovasc Dis* 2005, **15**(3):219-224.

20. Appelman Y, van Rijn BB, Ten Haaf ME, Boersma E, Peters SA: **Sex differences in cardiovascular risk factors and disease prevention**. *Atherosclerosis* 2015, **241**(1):211-218.

21. Liao B, Cheng K, Dong S, Liu H, Xu Z: **Effect of apolipoprotein A1 genetic polymorphisms on lipid profiles and the risk of coronary artery disease**. *Diagn Pathol* 2015, **10**:102.

22. Dawar R, Gurtoo A, Singh R: **Apolipoprotein A1 gene polymorphism (G-75A and C+83T) in patients with myocardial infarction: a pilot study in a north Indian population**. *Am J Clin Pathol* 2010, **134**(2):249-255.

23. Pitsavos C, Panagiotakos DB, Chrysohoou C, Stefanadis C: **Epidemiology of cardiovascular risk factors in Greece: aims, design and baseline characteristics of the ATTICA study**. *BMC Public Health* 2003, **3**:32.

24. Papathanasiou G, Georgoudis G, Papandreou M, Spyropoulos P, Georgakopoulos D, Kalfakakou V, Evangelou A: **Reliability measures of the short International**

Physical Activity Questionnaire (IPAQ) in Greek young adults. *Hellenic J Cardiol* 2009, **50**(4):283-294.

25. Panagiotakos DB, Tzima N, Pitsavos C, Chrysohoou C, Papakonstantinou E, Zampelas A, Stefanadis C: **The Relationship between Dietary Habits, Blood Glucose and Insulin Levels among People without Cardiovascular Disease and Type 2 Diabetes; The ATTICA Study**. *Rev Diabet Stud* 2005, **2**(4):208-215.

26. **Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus**. *Diabetes Care* 1997, **20**(7):1183-1197.

27. Ridker PM, Rifai N, Cook NR, Bradwin G, Buring JE: **Non–HDL Cholesterol, Apolipoproteins A-I and B100, Standard Lipid Measures, Lipid Ratios, and CRP as Risk Factors for Cardiovascular Disease in Women**. *JAMA* 2005, **294**(3):326-333.

28. Taskinen M-R, Barter PJ, Ehnholm C, Sullivan DR, Mann K, Simes J, Best JD, Hamwood S, Keech AC, investigators obotFs: **Ability of traditional lipid ratios and apolipoprotein ratios to predict cardiovascular risk in people with type 2 diabetes**. *Diabetologia* 2010, **53**(9):1846-1855.

29. Chrysohoou C, Panagiotakos DB, Pitsavos C, Antoniades C, Skoumas J, Brown M, Stefanadis C: **Evidence for association between endothelial nitric oxide synthase gene polymorphism (G894T) and inflammatory markers: the ATTICA study**. *Am Heart J* 2004, **148**(4):733-738.

30. Koloverou E, Panagiotakos DB, Pitsavos C, Chrysohoou C, Georgousopoulou EN, Pitaraki E, Metaxa V, Stefanadis C, Group AS: **10-year incidence of diabetes and associated risk factors in Greece: the ATTICA study (2002-2012)**. *The review of diabetic studies : RDS* 2014, **11**(2):181-189.

31. Panagiotakos DB, Georgousopoulou EN, Pitsavos C, Chrysohoou C, Metaxa V, Georgiopoulos GA, Kalogeropoulou K, Tousoulis D, Stefanadis C: **Ten-year (2002-2012) cardiovascular disease incidence and all-cause mortality, in urban Greek population: the ATTICA Study**. *Int J Cardiol* 2015, **180**:178-184.

32. Jeenah M, Kessling A, Miller N, Humphries S: **G to A substitution in the promoter region of the apolipoprotein AI gene is associated with elevated serum apolipoprotein AI and high density lipoprotein cholesterol concentrations**. *Mol Biol Med* 1990, **7**(3):233-241.

33. Kunutsor SK, Kieneker LM, Bakker SJL, James RW, Dullaart RPF: **Incident type 2 diabetes is associated with HDL, but not with its anti-oxidant constituent - paraoxonase-1: The prospective cohort PREVEND study**. *Metabolism* 2017, **73**:43-51.

34. Hanley AJG, Williams K, Gonzalez C, D'Agostino RB, Wagenknecht LE, Stern MP, Haffner SM: **Prediction of Type 2 Diabetes Using Simple Measures of Insulin Resistance: : combined results from the San Antonio Heart Study, the Mexico City Diabetes Study, and the Insulin Resistance Atherosclerosis Study.** *Diabetes* 2003, **52**(2):463-469.

35. Lyssenko V, Almgren P, Anevski D, Perfekt R, Lahti K, Nissén M, Isomaa B, Forsen B, Homström N, Saloranta C *et al*: **Predictors of and Longitudinal Changes in Insulin Sensitivity and Secretion Preceding Onset of Type 2 Diabetes**. *Diabetes* 2005, **54**(1):166-174.

36. Fizelova M, Miilunpohja M, Kangas AJ, Soininen P, Kuusisto J, Ala-Korpela M, Laakso M, Stancakova A: **Associations of multiple lipoprotein and apolipoprotein measures with worsening of glycemia and incident type 2 diabetes in 6607 non-diabetic Finnish men**. *Atherosclerosis* 2015, **240**(1):272-277.

37. Curtin A, Deegan P, Owens D, Collins P, Johnson A, Tomkin GH: **Elevated triglyceride-rich lipoproteins in diabetes. A study of apolipoprotein B-48**. *Acta Diabetol* 1996, **33**(3):205-210.

38. Ruano G, Seip RL, Windemuth A, Zollner S, Tsongalis GJ, Ordovas J, Otvos J, Bilbie C, Miles M, Zoeller R *et al*: **Apolipoprotein A1 genotype affects the change in high density lipoprotein cholesterol subfractions with exercise training**. *Atherosclerosis* 2006, **185**(1):65-69.

39. Taylor R: **Banting Memorial lecture 2012: reversing the twin cycles of type 2 diabetes**. *Diabet Med* 2013, **30**(3):267-275.

Table 1. Baseline lifestyle, biochemical variables and 10-year (2002-2012) incidence of diabetes, in the ATTICA study cohort (*n=1485*).

	Males *(n= 726)*	**Females *(n=759)***	***p***
New diabetes cases, n (%)	97 (13.4)	94 (12.4)	0.574
Age, years	46 ± 13	45 ± 14	0.458
Ever Smoker, n (%)	456 (62.9)	346 (45.6)	<0.001
Body mass index, kg/m^2	27.2 ± 3.6	25.3 ± 4.7	<0.001
Sedentary lifestyle, n (%)	408 (56.2)	452 (59.6)	0.191
Family history of diabetes, n (%)	135 (18.6)	156 (20.5)	0.416
Fasting glucose, mg/dl	91 ± 13	88 ± 12	<0.001
Fasting insulin, μU/ml	14.0 ± 3.5	11.8 ± 2.9	<0.001
HOMA-IR	3.1 ± 0.69	2.6 ± 0.59	<0.001
Total cholesterol, mg/dL	197 ± 41	191 ± 37	0.012
HDL-cholesterol, mg/dL	45 ± 15	53 ± 14	<0.001
LDL-cholesterol, mg/dL	126 ± 36	119 ± 38	0.001
Triglycerides, mg/dL	113 (79,160)	82 (59,117)	<0.001*
ApoA1, mg/dL	148 ± 23	163 ± 27	<0.001
ApoB, mg/dL	113 ± 29	100 ± 42	<0.001
ApoB/ApoA1	0.781 ± 0.232	0.641 ± 0.357	<0.001
ApoA1/HDL	3.47 ± 0.585	3.20 ± 0.762	<0.001
ApoB/LDL	0.928 ± 0.235	0.862 ± 0.265	<0.001
LDL/ApoA1	0.870 ± 0.283	0.755 ± 0.285	<0.001
TG/ApoA1	0.932 ± 0.630	0.599 ± 0.391	<0.001
TG/ApoB	1.18 ± 0.769	0.982 ± 0.850	<0.001

Data are presented as mean values and standard deviation for normally distributed variables and median (1st, 3rd quartile) for not normally distributed variables (*). Categorical variables are presented as absolute and relative frequencies. *p*-values derived from independent samples test for the normally distributed variables and Mann-Whitney test for the non-normally distributed variables (*) to test differences between genders and chi-square test was used for the categorical variables. HDL = high-density lipoprotein, LDL = low-density lipoprotein; HOMA-IR = homeostasis model assessment-insulin resistance; apoA1=apolipoprotein A-I; apoB=apolipoprotein B.

Table 2. Multivariable logistic regression models for various lipid biomarkers for the 10-year incidence of diabetes in the ATTICA study, stratified by gender *(n=1485)*.

	Males *(n=726)*		**Females *(n=759)***	
Variable	**OR**	**95% CI**	**OR**	**95% CI**
Model 1: ApoB (per 1 mg/dL)	1.01	0.99-1.02	1.01	0.99-1.01
Model 2: ApoA1 (per 1 mg/dL)	0.98	0.97-1.00	1.00	0.98-1.01
Model 3: ApoB/ApoA1 (per 1 unit/per 1 SD)	3.72/1.36	0.97-14.2/0.99-1.85	1.51/1.16	0.74-3.05/0.90-1.49
Model 4: ApoA1/HDL (per 1 unit/per 1 SD)	0.99/0.99	0.57-1.72/0.72-1.37	0.78/0.83	0.48-1.28/0.57-1.21
Model 5: ApoB/LDL (per 1 unit/per 1 SD)	4.03/1.39*	1.05-15.5/1.01-1.90	1.68/1.15	0.53-5.21/0.85-1.55
Model 6: LDL/ApoA1 ((per 1 unit/per 1 SD)	1.40/1.10	0.45-4.29/0.80-1.51	1.13/1.04	0.38-3.34/0.76-1.41
Model 7: TG/ApoA1 (per 1 unit/per 1 SD)	1.85/1.47*	1.20-2.87/1.12-1.94	1.56/1.19	0.80-3.05/0.92-1.55
Model 8: TG/ApoB (per 1 unit/per 1 SD)	1.17/1.13	0.86-1.57/0.89-1.42	1.07/1.06	0.81-1.39/0.84-1.32

OR: Odds Ratio; CI: Confidence Interval; apoB: apolipoprotein B; apoA1: apolipoprotein A-I; HDL: High-Density Lipoprotein –cholesterol; LDL: Low-Density Lipoprotein –cholesterol; TG: triglycerides. OR and CIs derived from multivariable binary logistic regression models adjusted for age, smoking status, physical activity status, HOMA-IR, family history of type 2 diabetes, BMI. (*) indicates Bonferroni corrected p-value significantly low.

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Table 3. Multivariable logistic regression models for various lipid and lipoprotein biomarkers, for the 10-year incidence of diabetes in the ATTICA study, stratified by apoA1-75G/A polymorphism *(n=304)*.

	GG *(n=215)*		**GA *(n=89)***	
Variable	**OR**	**95% CI**	**OR**	**95% CI**
Model 1: ApoB (per 1 mg/dL)	1.01	0.98-1.03	1.00	0.96-1.04
Model 2: ApoA1 (per 1 mg/dL)	0.98	0.96-1.01	0.99	0.96-1.03
Model 3: ApoB/ApoA1 (per 1 unit/per 1 SD)	1.50/1.13	0.25-8.87/0.65-1.98	0.80/0.93	0.01-60.1/0.24-3.59
Model 4: ApoA1/HDL (per 1 unit/per 1 SD)	0.75/0.82	0.29-1.94/0.42-1.59	0.76/0.83	0.14-4.06/0.26-2.65
Model 5: ApoB/LDL (per 1 unit/per 1 SD)	1.45/1.10	0.10-19.4/0.56-2.12	0.12/0.58	0.00-159/0-3.61
Model 6: LDL/ApoA1 (per 1 unit/per 1 SD)	2.10/1.24	0.11-38.4/0.53-2.87	2.11/1.24	0.02-162/0.32-4.35
Model 7: TG/ApoA1 (per 1 unit/per 1 SD)	2.11/1.50	0.74-5.96/0.85-2.64	1.02/1.01	0.25-4.03/0.47-2.13
Model 8: TG/ApoB (per 1 unit/per 1 SD)	1.86/1.66	0.75-4.62/0.79-3.50	2.57/2.16	0.43-15.4/0.50-9.36

OR: Odds Ratio; CI: Confidence Interval; apoB: apolipoprotein B; apoA1: apolipoprotein A-I; HDL: High-Density Lipoprotein –cholesterol; LDL: Low-Density Lipoprotein –cholesterol; TG: triglycerides. OR and CIs derived from various multivariable binary logistic regression models adjusted for gender, age, smoking status, physical activity status, HOMA-IR, family history of type 2 diabetes, BMI. (*) indicates Bonferroni corrected p-value significantly low.

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Highlights

- ApoA1 levels, ApoB:LDL and TG:apoA1 ratios are associated with 10-year risk of T2DM in males only
- In males only a unit change in apoB: LDL cholesterol increased risk of type 2 diabetes by 303%
- In males only a unit change in triglycerides: apoA1 increased risk of type 2 diabetes by 85%
- HOMA-IR predicted the 10-year incidence of T2DM only in apoA1 75 GG carriers
- Physical activity may moderate the influence of HOMA-IR on T2DM incidence only in carriers of apoA1 75 GG.